

AMENDMENT

Please cancel Claims 7-20 and 36-50, without prejudice, and amend Claims 21, 23-24, 26-33, 53-54, 56-59, 62-64, 66-67 and 69-73 as follows:

21. (Twice Amended) A method of detecting the presence of a predetermined target nucleic acid sequence in a sample, comprising the steps of:

(a) contacting, under conditions in which a FEN-1 polypeptide exhibits cleavage activity, a sample suspected of containing a target nucleic acid comprising the predetermined target sequence with:

(i) a 5'-polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region that is capable of specifically hybridizing under said cleavage conditions to a first portion of the target sequence and a 5'-region located immediately 5' to the 3'-region; and

(ii) a 3'-polynucleotide probe comprising a 5'-region that is capable of specifically hybridizing under said cleavage conditions to a second portion of the target sequence which is located immediately 3' to the first portion and a 3'-region located immediately 3' to the 5'-region,

such that the 3'-region of the 5'-probe and the 5'-region of the 3'-probe specifically hybridize immediately contiguously with one another to the first and second portions, respectively, of the target sequence to form a 5',3'-double flap structure cleavable by a FEN-1 polypeptide;

(b) cleaving the 5',3'-double flap structure with a FEN-1 polypeptide; and

(c) detecting the presence or absence of, and/or quantifying the amount of, FEN-1 polypeptide-generated cleavage, thereby detecting the presence of the target sequence in the sample.

23. (Amended) The method of Claim 22 in which the 5'-region of the 5'-probe contains the detectable label.

24. (Amended) The method of Claim 23 in which the 5'-end of the 5'-probe contains the detectable label.

26. (Amended) The method of Claim 21 in which the FEN-1 polypeptide is encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

27. (Amended) The method of Claim 26 in which the FEN-1 polypeptide is encoded by a polynucleotide comprising SEQ ID NO:28.

28. (Amended) The method of Claim 21 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

29. (Amended) The method of Claim 21 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

30. (Amended) The method of Claim 21 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

31. (Amended) The method of Claim 21 in which the 3'-region of the 3'-probe is 1 to 10 nucleotides in length.

32. (Amended) The method of Claim 21 in which the 3'-region of the 3'-probe is 1 nucleotide in length.

33. (Amended) The method of Claim 21 in which the 5'-region of the 5'-probe is 1 to 20 nucleotides in length.

53. (Amended) The hybridization complex of Claim 52 in which the 5'-region of the first probe contains the detectable label.

54. (Amended) The hybridization complex of Claim 53 in which the 5'-end of the first probe contains the detectable label.

56. (Amended) The hybridization complex of Claim 51 in which the 3'-region of the second probe is 1 to 10 nucleotides in length.

57. (Amended) The hybridization complex of Claim 56 in which the 3'-region of the second probe is 1 nucleotide in length.

58. (Amended) The hybridization complex of Claim 51 in which the 5'-region of the first probe is 1 to 20 nucleotides in length.

59. (Thrice Amended) A kit for use in detecting the presence of a predetermined target nucleic acid sequence in a sample, comprising:

(a) a FEN-1 polypeptide;

(b) a first polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region capable of specifically hybridizing under FEN-1 polypeptide cleavage conditions to a first portion of a the predetermined target sequence and a 5'-region located immediately 5' to the 3'-region; and

(c) a second polynucleotide probe comprising a 5'-region capable of specifically hybridizing under FEN-1 polypeptide cleavage conditions to a second portion of the target sequence which is located immediately 3' to the first portion and a 3'-region located immediately 3' to the 5'-region.

wherein the 3'-region of the first probe and the 5'-region of the second probe are capable of specifically hybridizing immediately contiguously with one another to the first and second portions, respectively, of the target sequence to form a 5',3'-double flap structure that is capable of being cleaved by the FEN-1 polypeptide.

62. (Amended) The kit of Claim 59 in which the 3'-region of the second probe is 1 to 10 nucleotides in length.

63. (Amended) The kit of Claim 59 in which the 3'-region of the second probe is 1 nucleotide in length.

64. (Amended) The kit of Claim 59 in which the 5'-region of the first probe is 1 to 20 nucleotides in length.

66. (Amended) The kit of Claim 65 in which the 5'-region of the first probe contains a the detectable label.

67. (Amended) The kit of Claim 66 in which the 5'-end of the first probe contains the detectable label.

69. (Amended) The kit of any one of Claim 59-68 in which the FEN-1 polypeptide is encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

70. (Amended) The kit of any one of Claims 59-68 in which the FEN-1 polypeptide is encoded by a polynucleotide comprising SEQ ID NO. 28.

71. (Amended) The kit of any one of Claims 59-68 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

72. (Amended) The kit of any one of Claims 59-68 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

73. (Amended) The kit of any one of Claims 59-68 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

APPENDIX B

Claims as Pending After Entry of Instant Amendment

1. An isolated polynucleotide encoding a FEN-1 polypeptide as shown in SEQ ID NO:1 or SEQ ID NO:3, or a fragment of said polypeptide having flap endonucleolytic cleavage activity.
2. An isolated polynucleotide, wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:29-51.
3. An isolated polynucleotide of Claim 2, wherein said polynucleotide comprises the sequence of SEQ ID NO:28.
4. A host cell comprising the polynucleotide of Claim 1.
5. A non-mammalian host cell comprising a mammalian FEN-1 polypeptide of Claim 1.
6. The polynucleotide of Claim 1 that is full length.
21. (Twice Amended) A method of detecting the presence of a predetermined target nucleic acid sequence in a sample, comprising the steps of:
 - (a) contacting, under conditions in which a FEN-1 polypeptide exhibits cleavage activity, a sample suspected of containing a target nucleic acid comprising the predetermined target sequence with:
 - (i) a 5'-polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region that is capable of specifically hybridizing under said cleavage conditions to a first portion of the target sequence and a 5'-region located immediately 5' to the 3'-region; and
 - (ii) a 3'-polynucleotide probe comprising a 5'-region that is capable of specifically hybridizing under said cleavage conditions to a second portion of the target sequence which is located immediately 3' to the first portion and a 3'-region located immediately 3' to the 5'-region,
 - such that the 3'-region of the 5'-probe and the 5'-region of the 3'-probe specifically hybridize immediately contiguously with one another to the first and second portions,

respectively, of the target sequence to form a 5',3'-double flap structure cleavable by a FEN-1 polypeptide;

(b) cleaving the 5',3'-double flap structure with a FEN-1 polypeptide; and

(c) detecting the presence or absence of, and/or quantifying the amount of, FEN-1 polypeptide-generated cleavage, thereby detecting the presence of the target sequence in the sample.

22. The method of Claim 21 in which the 5'-probe contains a detectable label.

23. (Amended) The method of Claim 22 in which the 5'-region of the 5'-probe contains the detectable label.

24. (Amended) The method of Claim 23 in which the 5'-end of the 5'-probe contains the detectable label.

25. The method of Claim 21 in which the 5'-probe is immobilized on a support.

26. (Amended) The method of Claim 21 in which the FEN-1 polypeptide is encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

27. (Amended) The method of Claim 26 in which the FEN-1 polypeptide is encoded by a polynucleotide comprising SEQ ID NO:28.

28. (Amended) The method of Claim 21 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

29. (Amended) The method of Claim 21 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

30. (Amended) The method of Claim 21 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

31. (Amended) The method of Claim 21 in which the 3'-region of the 3'-probe is 1 to 10 nucleotides in length.

32. (Amended) The method of Claim 21 in which the 3'-region of the 3'-probe is 1 nucleotide in length.

33. (Amended) The method of Claim 21 in which the 5'-region of the 5'-probe is 1 to 20 nucleotides in length.

34. The method of any one of Claims 21-33 in which the amount of FEN-1 polypeptide-generated cleavage is quantified.

35. The method of any one of Claims 21-33 in which the presence or absence of FEN-1 polypeptide-generated cleavage is detected.

51. A hybridization complex comprising:

(a) a bridge polynucleotide comprising a first portion and second portion located immediately 3' to the first portion;

(b) a first polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region and a 5'-region located immediately 5' to the 3'-region; and

(c) a second polynucleotide probe comprising a 5'-region and a 3'-region located immediately 3' to the 5'-region,

wherein the 3'-region of the first probe and the 5'-region of the second probe are specifically hybridized immediately contiguously with one another to the first and second portions, respectively, of the same bridge polynucleotide molecule, thereby forming a hybridization complex.

52. The hybridization complex of Claim 51 in which the first probe contains a detectable label.

53. (Amended) The hybridization complex of Claim 52 in which the 5'-region of the first probe contains the detectable label.

54. (Amended) The hybridization complex of Claim 53 in which the 5'-end of the first probe contains the detectable label.

55. The hybridization complex of Claim 51 in which the first probe is immobilized on a substrate.

56. (Amended) The hybridization complex of Claim 51 in which the 3'-region of the second probe is 1 to 10 nucleotides in length.

57. (Amended) The hybridization complex of Claim 56 in which the 3'-region of the second probe is 1 nucleotide in length.

58. (Amended) The hybridization complex of Claim 51 in which the 5'-region of the first probe is 1 to 20 nucleotides in length.

59. (Thrice Amended) A kit for use in detecting the presence of a predetermined target nucleic acid sequence in a sample, comprising:

(a) a FEN-1 polypeptide;

(b) a first polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region capable of specifically hybridizing under FEN-1 polypeptide cleavage conditions to a first portion of a the predetermined target sequence and a 5'-region located immediately 5' to the 3'-region; and

(c) a second polynucleotide probe comprising a 5'-region capable of specifically hybridizing under FEN-1 polypeptide cleavage conditions to a second portion of the target sequence which is located immediately 3' to the first portion and a 3'-region located immediately 3' to the 5'-region,

wherein the 3'-region of the first probe and the 5'-region of the second probe are capable of specifically hybridizing immediately contiguously with one another to the first and second portions, respectively, of the target sequence to form a 5',3'-double flap structure that is capable of being cleaved by the FEN-1 polypeptide.

60. The kit of Claim 59 further in which the first or second probe contains a detectable label.

61. The kit of Claim 59 in which the FEN-1 polypeptide contains a detectable label.

62. (Amended) The kit of Claim 59 in which the 3'-region of the second probe is 1 to 10 nucleotides in length.

63. (Amended) The kit of Claim 59 in which the 3'-region of the second probe is 1 nucleotide in length.

64. (Amended) The kit of Claim 59 in which the 5'-region of the first probe is 1 to 20 nucleotides in length.

65. The kit of Claim 59 in which the first probe contains a detectable label.

66. (Amended) The kit of Claim 65 in which the 5'-region of the first probe contains a the detectable label.

67. (Amended) The kit of Claim 66 in which the 5'-end of the first probe contains the detectable label.

68. The kit of Claim 59 in which the first or second probe is immobilized on a substrate.

69. (Amended) The kit of any one of Claim 59-68 in which the FEN-1 polypeptide is encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

70. (Amended) The kit of any one of Claims 59-68 in which the FEN-1 polypeptide is encoded by a polynucleotide comprising SEQ ID NO. 28.

71. (Amended) The kit of any one of Claims 59-68 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

72. (Amended) The kit of any one of Claims 59-68 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

73. (Amended) The kit of any one of Claims 59-68 in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.